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IMPACT OF ORGANIC LIQUID FORMULATION, JEEVAMRUTHA ON PHOTOSYNTHETIC PIGMENTS OF *OCIMUM BASILICUM* L. (SWEET BASIL) UNDER NACL INDUCED SALINITY STRESS

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Abstract

Salinity is one of the most important abiotic stresses which affects metabolism and ultimately causes reduction in the productivity of plants. The present investigation was carried out to access the effect of four different NaCl concentrations viz. 25, 50, 75 and 100 mM on photosynthetic pigments i.e. chlorophyll 'a', 'b' and carotenoid content in the leaves of *Ocimum basilicum* L. (Sweet Basil) with and without treatment of organic liquid formulation, jeevamrutha. The results showed that NaCl had adversely affected and decreases, chlorophyll 'a', 'b' and carotenoid content with the increasing concentrations, moreover organic liquid formulation; jeevamrutha, enhances the content of chlorophyll 'a', 'b' and carotenoid in the treated plants. The outcomes of the present study suggested that organic liquid formulation; jeevamrutha had potential to help the plants, to overcome from the stress conditions, its eventually supports, proper growth and development by supplying different essential nutrients and also by beneficial microbes that enhances the fertility as well as maintains better health of soil.

Keywords: NaCl, chlorophyll, sweet basil, organic liquid formulation, carotenoid.

Introduction

Salinity is one of the main environmental factors that determine the productivity and distribution of plants. The problems related to salinity is rapidly increasing throughout the world, declining average yield for most of the major crop plants (Bray *et al.,* 2000). The salinity particularly, dominated with NaCl is one of the factors of soil that negatively affects the growth as well as the yield of plants (Wang *et al.,* 2004). Due to salt accumulation every year more and more lands are becoming non-productive. Therefore, it is extremely essential to understand the mechanisms of plant tolerance to salinity stress Bartels and Sunkar (2005). Salinity is the major aspect that interrupts the process of photosynthesis in the plants. The main cause for reduction in the process of photosynthesis under saline conditions is not only by stomata closure that leads to the reduction of intercellular CO_2 concentration, but also by non-stomata factors. It was evident that salt stress affects photosynthetic enzymes as well as chlorophyll and carotenoids content in plants (Stepien and Klobus, 2006). The problem of salinity is enormously severe that causes different types of damages to plants so it is particularly, very necessary to check the responses of plants under saline conditions. Indian basil (*Ocimum basilicum* L.) belongs to the family Lamiaceae, is a very common plant growing in India and it is a chief source of essential oil and aromatic chemicals for many industrially purposes. The essential oil extracted from sweet basil plants is extremely valuable and used in different types of perfumes, aromatherapy, flavoring liquors, fly repellant, and in a range of medicines (Bahl *et al.,* 2000). So the study focuses on the changes in chlorophyll 'a', 'b' and carotenoid content of *Ocimum basilicum* L. (Sweet Basil) under different levels of NaCl stress with and without the treatment of organic liquid formulation, jeevamrutha. Further, the use of various organic manures in agriculture is established to be significant because it maintains the fertility of soil and also helps in enhancement of growth and development of plants. The organic liquid formulation, jeevamrutha also comes in one of the low cost formulations, which are responsible for the enrichment of soil with indigenous micro organisms required for better mineralization of soil and it helps in enhancement of growth of plants (Gore and Sreenivasa, 2011). Therefore, the present study was mainly designed to check the fluctuations in photosynthetic pigments like chlorophyll 'a', 'b' and carotenoid of *Ocimum basilicum* L. (Sweet Basil) under NaCl induced salinity stress and its mitigation by organic liquid formulation, jeevamrutha.

Materials and Methods

The present study was conducted in two trials, in the years 2015 and 2016 at Department of Biological Sciences, SHUATS, Prayagraj (Allahabad). The plants of *Ocimum basilicum* L. (Sweet Basil) variety CIM-Saumya were sown in pots with 5 kilograms of soil. The treatment of NaCl (Sodium Chloride) was applied to the soil directly and the treatment of organic liquid formulation, jeevamrutha was applied by irrigation water. The experiment was conducted with four levels of NaCl i.e. 25, 50, 75 and 100 mM per kg soil, with and without treatment of organic liquid formulation, jeevamrutha. After 90 days of experiment, leaves samples were collected for further analysis. The experimental data was analyzed statistically by two-way ANOVA and the values in the tables shows the mean values of three different replicates.

Method used for preparation of jeevamrutha: Organic liquid formulation, jeevamrutha was prepared according to the method recommended by Palekar, (2006). The ingredients were as follows, 10 kg cow dung, 10 liter cow urine, two kg jaggery, two kg of gram or any pulse flour, hand full of rhizospheric soil, 200 liter water. All these materials were mixed well in a container with the help of wooden stick. The mixture was stirred regularly twice a day and kept for fermentation for 5 to 7 days. The prepared liquid formulation was used as soil application, by applying through irrigation water.

Estimation of chlorophyll 'a' and 'b':

The chlorophyll content was estimation by the method given by Arnon, (1949). 100 mg of fresh leaves were grounded with pestle and mortar. 10 ml of 80 % acetone was added to it and then the samples were taken in test tubes and were kept overnight. Next to that the samples were homogenized at 3000 RPM for 15 minutes. Finally the absorbance of supernatant was recorded at 645 nm and 663 nm respectively to get the values of chlorophyll 'a' and 'b' content.

Estimation of carotenoid

Carotenoid content was determined according to the method of Lichtenthaler and Welburn, (1983). 100 mg of fresh leaves were taken and crushed in 80% acetone and the volume was made to 10 ml with 80 % acetone. Then centrifuge at 800 RPM for 5 minutes. The reading of supernatant was recorded under 470 nanometer to get the content of carotenoid in the samples.

Results and Discussion

The result for chlorophyll 'a' content was presented in (Table 1.) it reveals that NaCl significantly influences the chlorophyll 'a' content in all the treatments. Gradual decline was recorded in chlorophyll 'a' content with the increasing concentration of NaCl among the treatments in both the trials. Further the treatment of jeevamrutha significantly increases chlorophyll 'a' content in the treated plants. The maximum chlorophyll 'a' content was recorded in the lowest treatment of NaCl with jeevamrutha in NaCl25+J.

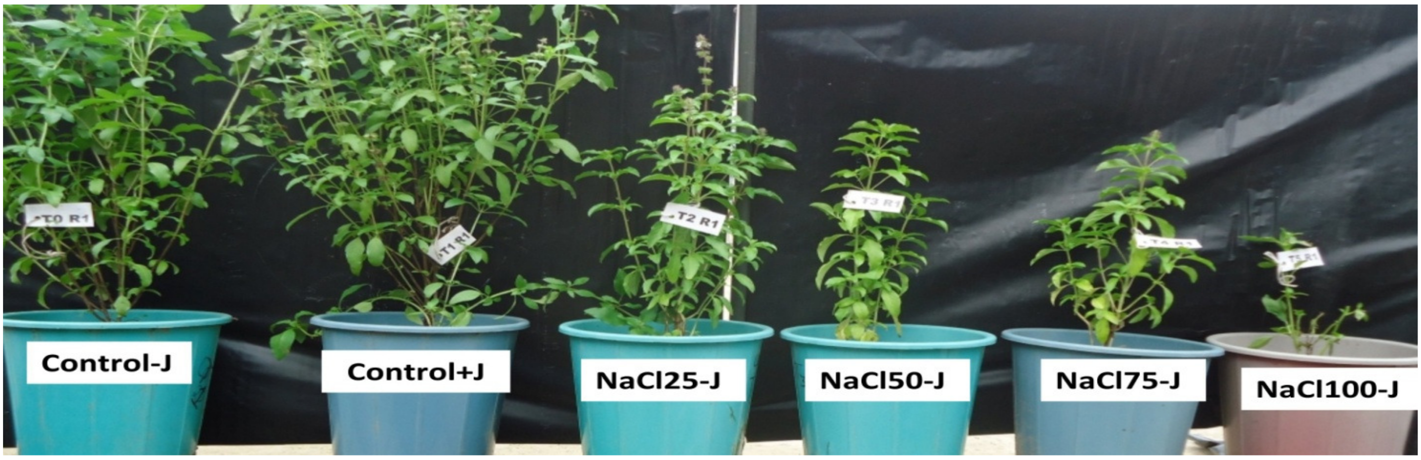

Plate 1 : Effect of different concentrations of NaCl (25 mM, 50mM, 75mM and 100mM) on sweet basil plants in the absence (-J) of jeevamrutha

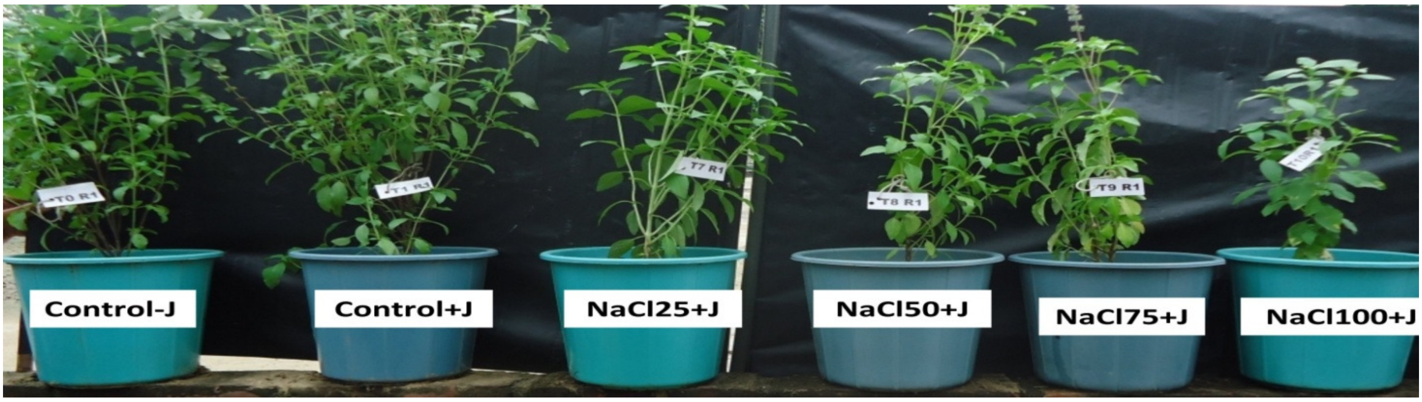

Plate 2 : Effect of different concentrations of NaCl (25 mM, 50mM, 75mM and 100mM) on sweet basil plants in the presence (+J) of jeevamrutha

Table 1 : Effect of different concentrations of NaCl (25 mM, 50mM, 75mM and 100mM) on chlorophyll 'a' content (mg gm^{-1} fresh weight) of sweet basil plants in the presence (+J) and absence (-J) of jeevamrutha

Chlorophyll 'a' (mg gm^{-1} fresh weight)		
Treatments	**I Trial**	**II Trial**
Control- J	2.967	2.982
Control +J	2.997	3.016
NaCl25-J	2.971	2.986
NaCl50-J	2.850	2.872
NaCl75-J	2.743	2.766
NaCl100-J	2.653	2.686
NaCl25+J	2.993	3.012
NaCl50+J	2.880	2.903
NaCl75+J	2.766	2.792
NaCl100+J	2.691	2.717
F Test	S	S
CD (0.05%)	0.016841	0.018168
S. Em. (±)	9.64E-05	0.000112

*–J = without jeevamrutha, +J= with jeevamrutha

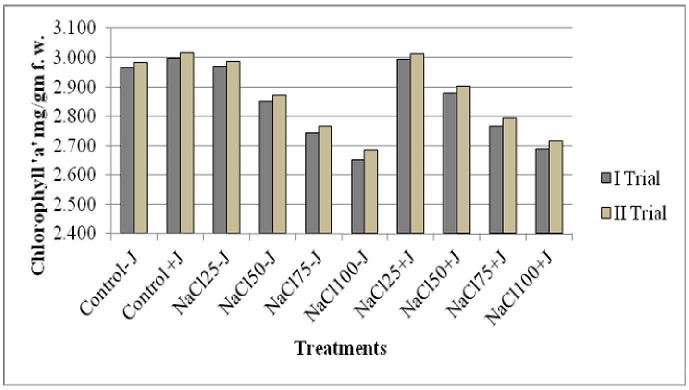

Fig. 1 : Effect of different concentrations of NaCl (25 mM, 50mM, 75mM and 100mM) on chlorophyll 'a' content (mg gm^{-1} fresh weight) of sweet basil plants in the presence (+J) and absence (-J) of jeevamrutha

In the same way chlorophyll 'b' content reduces with the linear increment in concentrations of NaCl. The result presented in (Table 2.) shows decrement in the level of chlorophyll 'b' with the increasing concentrations of NaCl in all the treatments. The plants treated with jeevamrutha showed slight increment in chlorophyll 'b' content. The maximum chlorophyll 'b' content was recorded in lowest treatment of NaCl with jeevamrutha in NaCl25+J in both the trials.

Table 2 : Effect of different concentrations of NaCl (25 mM, 50mM, 75mM and 100mM) on chlorophyll 'b' content (mg gm^{-1} fresh weight) of sweet basil plants in the presence (+J) and absence (-J) of jeevamrutha

Chlorophyll 'b' (mg gm^{-1} fresh weight)		
Treatments	**I Trial**	**II Trial**
Control- J	1.777	1.786
Control +J	1.795	1.802
NaCl25-J	1.782	1.790
NaCl50-J	1.703	1.717
NaCl75-J	1.648	1.653
NaCl100-J	1.586	1.610
NaCl25+J	1.786	1.796
NaCl50+J	1.721	1.735
NaCl75+J	1.653	1.673
NaCl100+J	1.608	1.628
F Test	S	S
CD (0.05%)	0.018434	0.012802
S. Em. (±)	0.000115	5.56954E-05

*–J = without jeevamrutha, +J= with jeevamrutha

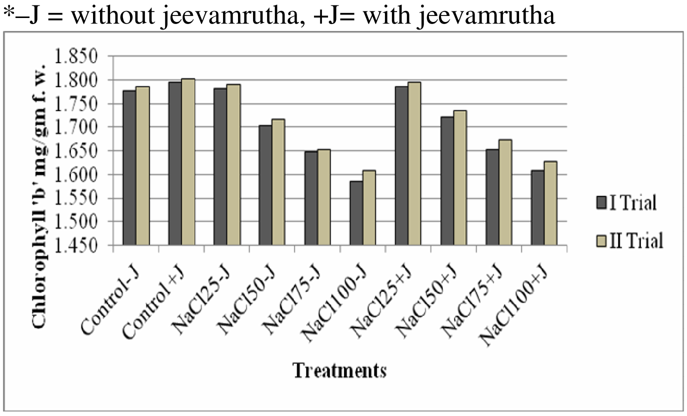

Fig. 2 : Effect of different concentrations of NaCl (25 mM, 50mM, 75mM and 100mM) on chlorophyll 'b' content (mg gm^{-1} fresh weight) of sweet basil plants in the presence (+J) and absence (-J) of jeevamrutha

Similarly, the result for carotenoid content presented in (Table 3.) revealed that carotenoid content was also negatively influenced by the treatments; gradual reduction in carotenoid content was recorded with rising concentrations of NaCl. The treatment of jeevamrutha showed slight increment in carotenoid content in comparison to untreated plants. The maximum carotenoid content was recorded in the least concentration of NaCl with jeevamrutha in NaCl25+J in both the trials.

Table 3 : Effect of different concentrations of NaCl (25 mM, 50mM, 75mM and 100mM) on carotenoid content (mg gm^{-1} fresh weight) of sweet basil plants in the presence (+J) and absence (-J) of jeevamrutha

Carotenoid content (mg gm^{-1} fresh weight)		
Treatments	**I Trial**	**II Trial**
Control- J	1.498	1.484
Control +J	1.650	1.630
NaCl25-J	1.584	1.553
NaCl50-J	1.558	1.529
NaCl75-J	1.407	1.368
NaCl100-J	1.366	1.349
NaCl25+J	1.585	1.560
NaCl50+J	1.565	1.541
NaCl75+J	1.410	1.373
NaCl100+J	1.370	1.357
F Test	S	S
CD (0.05%)	0.111766	0.112161
S. Em. (±)	0.004245	0.004275

*–J = without jeevamrutha, +J= with jeevamrutha

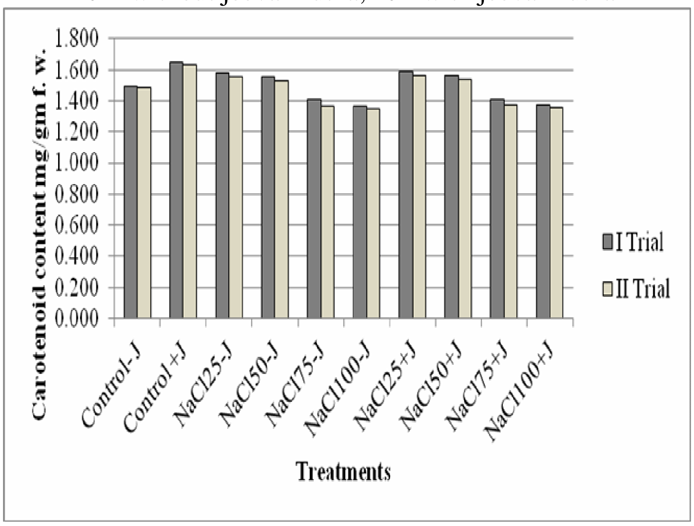

Fig. 3 : Effect of different concentrations of NaCl (25 mM, 50mM, 75mM and 100mM) on carotenoid content (mg gm^{-1} fresh weight) of sweet basil plants in the presence (+J) and absence (-J) of jeevamrutha

Many workers reported reduction in chlorophyll 'a', 'b' and carotenoid content under salinity stress as Akça and Samsunlu, (2012) reported reduction in chlorophyll a, chlorophyll b content with the increasing salt concentrations. There were significant differences between irrigation water salinity levels in chlorophyll a and chlorophyll b content in three walnut cultivars. Shah *et al.* (2017) also reported that increasing salinity significantly reduced leaf chlorophyll and carotenoid content in wheat (*Triticum aestivum* L.). They concluded that plant photosynthetic pigment content per-leaf area was intensely affected by salinity and nutrient stress.

The similar results were observed by Chandramohanan *et al.* (2014); Saravanavel *et al.* (2011). The carotenoid content of leaves were also affected by salinity stress, earlier workers also reported the same in their studies as Akcin and Yalcin, (2016) investigated the effects of salinity stress on photosynthetic pigments in *Salicornia prostrata* and *Suaeda prostrata* in natural habitats. They observed that increasing soil salinity decreases the carotenoid contents in both the taxa. Miladinova *et al.* (2013) also evaluated the effect of salt stress on growth parameters, chlorophyll a, chlorophyll b and carotenoids content in *Paulownia* clones. They concluded that carotenoid content significantly decreased in the leaves of EK and T2 clones of *Paulownia* grown in the highest concentrations of NaCl. The results were in agreement with the earlier reports by Vasudevan and Manickavasagam, (2018); Amirjani (2010). However, slight increment in chlorophyll 'a', 'b' and carotenoid content in presence of organic liquid formulation, jeevamrutha may be due to the presence of different nutrients, beneficial microbes in it that eventually supports the growth of plants. The reports related to increment in photosynthetic pigments due to different biofertilizers were also observed in earlier studies like El-Quesni *et al.* (2013) showed that compost significantly affected the photosynthetic pigments content of *Jatropha curcas* L. leaves. The study also emphasized that chlorophyll a, chlorophyll b and total carotenoids were significantly increased when seedlings treated with algae, phosphorien, microbien and compost. Similarly, Manjunatha *et al.* (2009) also reported the effect of farm yard manure treated with jeevamrut on soil properties and yield of sunflower. Likewise, they found that the application of jeevamrut increased the activity of microbes by solubilization and also enhances the uptake of nutrients. Gore and Sreenivasa, (2011) also reported significant increase in plant height and root length with the application of recommended dose of fertilizer + beejamrut + panchagavya + jeevamrut in their study. The similar results were also observed by Rakesh *et al.* (2017); Dinu *et al.* (2015). The possible reason for reduction in photosynthetic pigments under salt stress is considered to be an outcome of slow synthesis or fast breakdown of pigments in the cells Ashraf, (2003). On the other hand the decrease in chlorophyll concentration under saline conditions could be the attribute of amplified activity of enzyme chlorophyllase, which is responsible for chlorophyll degrading Reddy and Vora, (1986). Additionally, the decrease in carotenoids under salinity stress indicates photoinhibition due to the damage of photosystem. Moreover, the changes in the chlorophyll and carotenoids content might be the indication of oxidative stress Grzeszczuk *et al.* (2018).

Conclusion

The overall study concluded that chlorophyll 'a', 'b' and carotenoid content reduces with the increasing concentration of NaCl and further jeevamrutha treated plants showed increment in the values of chlorophyll 'a', 'b' and carotenoid respectively. The study suggested that organic liquid formulation, jeevamrutha is potent enough to help the plants in proper growth and development under NaCl induced stress conditions.

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